IN THE SPECIFICATION:

Please amend paragraph number **[0001]** as follows:

[0001] Related Applications: This application is a continuation of Application No. 09/516,307 filed on March 1, 2000, now U.S. Patent 6,316,274, issued November 13, 2001, which is a divisional of Application No. 08/979,582 filed on November 26, 1997, abandoned, which is a continuation-in-part of U.S. Patent Application Serial No. 08/748,687, filed on November 13, 1996, now U.S. Patent 5,919,712, issued July 6, 1999, which is a divisional of U.S. Patent Application Serial No. 08/263,522, filed June 22, 1994, now U.S. Patent No. 5,677,196, issued October 14, 1997, which is a continuation-in-part of Application No. 08/110,169, filed August 20, 1993, now U.S. Patent No. 5,516,703, issued May 14, 1996, and Application No. 08/071,579, filed June 2, 1993, now abandoned, which is a continuation-in-part of Application No. 08/064,608, filed May 18, 1993, now U.S. Patent 5,512,492, issued April 30, 1996.

Please amend paragraph number **[0009]** as follows:

[0009] It is further desirable for speed and convenience in "routine" testing, for example, testing of blood bank samples for viral antibodies, to have an evanescent immunofluorescent biosensor which is disposable and which provides multi-sample measurement capability. ~~Multi-sample~~ Multi-sample capability would allow a test sample and a control sample (such as a blank, a positive control, or, for a competition-type assay, a sample preloaded with tracer molecules) to be simultaneously illuminated and measured. Simultaneous multi-sample capability would also speed up the process of analyzing multiple samples and would reduce the effects of variation in the level of exciting light which are known to occur with typical light sources. However, in a typical prior art evanescent light device such as that of Block et al., U.S. Patent No. 4,909,990 issued March 20, 1990, the waveguide is a fiber optic rod whose shape makes it difficult to build a multi-well biosensor.

Please amend paragraph number **[0013]** as follows:

[0013] Another problem relates to the levels of nonspecific binding to the ~~antibody-coated~~ antibody-coated surface of the optical substrate. These levels are often sufficiently high to make detection of analyte at concentrations below about 10^{-10} M very difficult. Nonspecific binding can be reduced by including a wash step after the sample is incubated with the coated substrate to remove unbound tracer molecules. However, as discussed above, a wash step is undesirable. Second, nonspecific binding can be a serious problem unless the surface is "passivated" with a masking agent such as bovine serum albumin or with a thin coating of hydrophilic polymer such as poly(ethylene glycol) or poly(methacrylate). Without such passivation (which introduces yet another step into the procedure), nonspecific binding can be 50% or more of the specific binding. Even with passivated surfaces, nonspecific binding can be sufficient to reduce detection sensitivity and reproducibility.

Please amend paragraph number **[0016]** as follows:

[0016] The apparatus includes a biosensor comprising a planar waveguide having a receiving region on its edge for receiving light to be internally propagated. A semicylindrical lens is integrally adapted to the waveguide edge adjacent the receiving region, and at least one of the waveguide surfaces has a plurality of capture molecules immobilized thereon. The capture molecules may include a plurality of species, each configured to specifically bind a different analyte, and different species may be localized in different and mutually exclusive regions on the waveguide surface. In a highly preferred embodiment, the semicylindrical lens and the waveguide are integrally molded of an optical plastic, and the lens is oriented to aim the beam at a selected angle to the plane of the waveguide, the selected angle being less than the critical angle of reflection at the ~~waveguide-liquid~~ waveguide-liquid interface. The waveguide also may have a serrated portion forming the portion of the edge opposite the receiving region.

Please amend paragraph number **[0018]** as follows:

[0018] The invention further encompasses methods for site-specifically immobilizing the capture molecules to the waveguide surface, methods for coating silica and polystyrene waveguide surfaces to reduce nonspecific binding, methods of patterning a waveguide surface with patches of different capture molecule species using photo-affinity crosslinking agents, and waveguides prepared by any of these methods singly or in combination. The method for patterning the waveguide with patches of different capture molecules involves localized irradiation of one or more regions of the waveguide surface in the presence of a photo-affinity crosslinking agent. In a highly preferred method, the waveguide surface is coated with a coating agent which inhibits nonspecific protein binding to less than 5%-10%, and preferably as low as 1% to 2%, of nonspecific binding. Also preferably, the capture molecules are coupled to the waveguide in a site-specific manner. For ~~site-specific~~ site-specific coupling, Fab' fragments are preferred as these are easily prepared with thiol sites, the thiol sites being highly suitable for the surface coupling chemistry. The preferred method of ~~photo-activated~~ photo-activated crosslinking agent also utilizes thiol sites on the capture molecules. The site-specific coupling chemistry provides waveguide surfaces having 50% to 70% of the capture molecules with analyte binding sites readily available for binding.

Please amend paragraph number **[0035]** as follows:

[0035] FIGS. 10A-D are charts depicting data from a displacement fluoroimmunoassay performed with the ~~apparatus.~~ apparatus;

Please amend paragraph number **[0041]** as follows:

[0041] FIGS. 14A and 14B are a side view diagram of an improved imaging ~~photo-detection~~ photo-detection system and a top view of a photodiode array for use in the ~~system;~~ system, respectively;

Please amend paragraph number **[0043]** as follows:

[0043] FIG. 16 is a side view of a section of the waveguide surface ~~showing,in~~ showing, in schematic form, steps in a process of patterning a waveguide surface with different Fab' species;

Please amend paragraph number **[0050]** as follows:

[0050] ~~Light~~ Referring again to FIG. 1, light detection means, indicated generally at 150, are positioned to detect fluorescent light emitted from biosensor 120. The emitted light is reflective of the concentration of a selected analyte in a sample, as is better described subsequently in reference to FIGS. 2 and 7-10. Light detection means 150 includes a collection lens 152 positioned to collect the emitted fluorescence from a plane parallel to and displaced from the surface of optical substrate 122.

Please amend paragraph number **[0052]** as follows:

[0052] Detection means 150 may be any type of photodetector useful to detect light in the wavelength region spanning the wavelength range of the emitted fluorescence, as known in the art. However, in a preferred embodiment for simultaneous multi-analyte assays, detection means 150 is an imaging-type detector providing direct imaging of each of the fluorescent signal(s) originating in the evanescent zone 240, which is also referred to herein as an "excitation zone," as fluorescent molecules within the evanescent zone 24 are excited into a fluorescent state. In the apparatus of FIG. 1, detection means 150 is a CCD (charge-coupled device) detector which produces a signal like that depicted in FIG. 4C. Such imaging signal collection provides simultaneous measurement of multiple samples in a much simpler way than a system in which a separate optical element is needed to read each well or patch. The present imaging detection system also provides for collection of emitted fluorescence directly from the evanescent zone 240 (FIG. 2), rather than via evanescent penetration and propagation of the fluorescence in the waveguide.

Please amend paragraph number **[0057]** as follows:

[0057] As is better seen in FIG. 2, optical substrate 122 is embodied as a planar waveguide having at least one planar surface 200 spaced from a second surface 201 by a width 202. At least planar surface 200 is disposed in contact with a sample solution 203. A plurality of capture molecules 204 are immobilized on surface 200. The sample solution contains a plurality of analyte molecules 210 of a selected analyte and a plurality of tracer molecules 220. The capture molecules are chosen or constructed to bind to a binding moiety present on each of the analyte molecules 210. The tracer molecule 220 is chosen or constructed to emit fluorescent light in response to stimulation by light of the appropriate wavelength. The level of fluorescence emitted by the tracer molecules 220 is a measure of the amount of analyte bound to the capture molecule and is thereby reflective of the concentration of analyte molecules 210 in the solution.

Please amend paragraph number **[0059]** as follows:

[0059] Capture molecules 204 may be whole antibodies, antibody fragments such as Fab' fragments, whole antigenic molecules (haptens) or antigenic fragments, and oligopeptides which are antigenic and/or similar in three-dimensional conformation to an antibody-binding epitope. Capture molecules 204 may also be a receptor molecule of the kind usually found on a cell or organelle membrane and which has specificity for a desired analyte, or a portion thereof carrying the ~~analyte-specific-binding~~ analyte-specific-binding property of the receptor.

Please amend paragraph number **[0067]** as follows:

[0067] Gasket 320 is preferably made of a semirigid material having an index of refraction less than that of the waveguide material in the wavelength range of the exciting light. For the best results, it is believed that the index of refraction of the gasket material should be as low as possible compared to that of the waveguide. For a waveguide made of quartz or glass, the index of refraction would typically be from about 1.46 to 1.52, higher for high-lead glass. A transparent ~~(non-~~

~~pigmented)~~ (non-pigmented) silicon rubber (siloxane polymer) with an index of refraction of 1.35-1.43 is a presently preferred material for gasket 320. TEFLON-type materials such as PTFE (polytetrafluoroethylene) or FEP (fluorinated ethylene propylene) have indices of refraction of around 1.34-1.35 and may also be suitable. However, because TEFLON surfaces tend to adsorb protein in a nonspecific manner, silicon rubber is generally preferred.

Please amend paragraph number **[0075]** as follows:

[0075] FIGS. 5A-5D depict an alternate embodiment of a biosensor useful with the apparatus of FIG. 1. The biosensor indicated generally at 500 has an integrally mounted or formed focusing lens 502 and waveguide 504 arranged such that focusing lens 502 focuses light onto the forward end 506 of the waveguide 504. Focusing lens 502 is configured and positioned to focus a light beam 102 onto the forward end 506 of the waveguide 504 (FIGS. 5A, 5C). ~~Side walls~~ Sidewalls 511, 512, top and bottom walls 516, 517, and a removably sealing rear wall 518 enclose the space about the waveguide 504 to create reservoirs 520, 522.

Please amend paragraph number **[0079]** as follows:

[0079] The biosensor including the lens may be formed by molding of a suitable optical plastic. A holder comprising the reservoir walls, the lens, and frame elements as needed may be ~~pre-molded.~~ pre-molded. A silica-surface waveguide is inserted subsequently with a refractive-index-matched adhesive to secure it in place and seal it as needed to create separate channels. Alternatively, the holder may be molded with a silica-surface waveguide in place, thereby eliminating the need for the adhesive.

Please amend paragraph number **[0083]** as follows:

[0083] While the curved edge 34 of lens 14 is shown as being substantially ~~a semiright-cylinder~~ semiright-cylinder in shape, other lens shapes are possible as described previously herein with respect to FIGS. 3A and 5C.

Please amend paragraph number **[0087]** as follows:

[0087] In another improvement, the sheet excitation beam is arranged to enter the receiving edge of the waveguide at an angle to the plane of the waveguide. FIG. 13 shows an angled integral lens 670 configured to accept such angled beam entry. For this purpose, the beam originating from the laser should be shaped to a sheet of a width approximating the width of the receiving region of the waveguide and of relatively narrow thickness (preferably no more than ~~ten-fold,~~ ten-fold, and preferably one- to four-fold the waveguide thickness), using cylindrical and/or spherical lenses as known in the art.

Please amend paragraph number **[0089]** as follows:

[0089] FIG. 19 shows data for the fluorescence intensity of biotin-conjugated Cy5 dye bound to an avidin-coated silica waveguide (ch. A) as a function of beam entry angle, as compared to the "background" level of fluorescence from Cy5 dye free in solution (not conjugated to biotin and therefore not bound to the avidin-coated surface). The critical angle for TIR at the ~~silica-aqueous~~ silica-aqueous solution boundary is about 26°. The data of FIG. 19 indicate that the signal-to-background ratio (ch.A ÷ ch.B) for a beam entry angle of 20° to 25° is about 4-fold higher than for 0°. Thus, beam entry angles of one to about five degrees less than the critical angle are presently preferred for silica waveguides. For a polystyrene waveguide with an aqueous adjacent medium, the critical angle for TIR is about 33°, and a useful range of beam entry angles is from about 25° to about 32°, with the higher angles generally being preferable.

Please amend paragraph number **[0091]** as follows:

[0091] The following examples detail several methods for attaching the capture molecules to the waveguide surface in a site-specific manner. The general scheme for reducing the level of nonspecific binding is to coat the waveguide with a protein-resistant material and then immobilize the antibody to the coating. The scheme further includes derivatizing of the ~~protein-resistant~~ protein-resistant coating combined with site-specific modification of the antibody or

other capture molecule to be immobilized, so as to provide site-specific attachment of the capture molecule to the coating.

Please amend paragraph number **[0095]** as follows:

[0095] Antibodies could be coupled to this hydrogel in at least two ways. In one method, the carbohydrate groups in the Fc antibody region are oxidized to aldehydes by treatment with sodium metaperiodate. However, few antigen-binding fragments contain carbohydrate moieties useful for this purpose. Thus, a preferred method comprised modifying the pendant hydrazido groups of the hydrogel to a maleimido group by treatment with succinimidyl ~~4-(N-maleimido-methyl)cyclo-hexane-1-carboxylate~~ 4-(N-maleimido-methyl)cyclo-hexane-1-carboxylate (abbreviated SMCC; Pierce Chemicals). These maleimido groups can be reacted with the free thiol groups typically found in the C-terminal region of Fab' fragments, thereby coupling the Fab' fragments to the hydrogel.

Please amend paragraph number **[0104]** as follows:

[0104] This strategy was designed to exploit the very strong binding affinity of biotin for avidin (binding constant of around 10^{-15}). An avidin coating was readily made by physical adsorption on a silica surface. The Fab' fragments were then conjugated with biotin to form biotin-Fab' conjugates, also referred to as biotinylated Fab' fragments or b-Fab' fragments. The biotin is coupled at specific location(s) on the Fab' fragments. The avidin coated surface is then treated with the ~~b-Fab'~~ b-Fab' fragments, so that the biotin binds to the avidin, thereby immobilizing the Fab' fragment to the surface in a site-specific manner.

Please amend paragraph number **[0107]** as follows:

[0107] An alternate method was used for biotinylating whole antibodies, in which ~~biotin-LC-hydrazide~~ biotin-LC-hydrazide was coupled to oxidized carbohydrate groups in the Fc region of the antibody. Mab, designated 9-40 (a murine monoclonal IgG_1 antibody that binds

fluorescein), was oxidized by incubation at a concentration of 1-2 mg/ml protein in 10 mM sodium periodate, 0.1 M sodium acetate, pH 5.5, for 20 minutes at about 0°C. Glycerol was then added to a final concentration of 15 mM to quench the reaction, and the mixture incubated a further 5 minutes at 0°C. The oxidized Mab 9-40 was purified by gel filtration chromatography on Sephadex G25 equilibrated with 0.1M sodium acetate buffer, pH 5.5, and then reacted with 5 mM biotin-LC-hydrazide for 2 hours at room temperature with agitation. Unreacted biotin-LC-hydrazide was removed using a Sephadex G25 column equilibrated in PBS.

Please amend paragraph number **[0110]** as follows:

[0110] In this method, the terminal hydroxyl groups of polyethylene glycol (abbreviated PEG) were converted to primary amine or hydrazide groups by reaction with ethylenediamine (abbreviated ED) or hydrazine (abbreviated HZ), respectively, to produce PEG-ED_2 or PEG-HZ_2. The PEG molecules so modified were then coupled to APS-glutaraldehyde activated silica surfaces. One ED moiety on each PEG-ED_2 molecule couples to a free aldehyde group on the ~~silanized-glutaraldehyde-treated~~ silanized-glutaraldehyde-treated waveguide surface. The other ED (or HZ, if PEG-HZ_2 is used) is then available to bind to an aldehyde moiety in a capture molecule (binding protein) such as an oxidized antibody or antibody fragment.

Please amend paragraph number **[0117]** as follows:

[0117] Absolute antigen binding was determined by means of radiolabeled tracers or capture molecules. For example, radiolabeled BSA-FL_9 was allowed to be coupled with immobilized Fab' fragments for 5 or 60 minutes in phosphate buffer, pH 7.3, at room temperature. The tracer concentration was $1.5x10^{-7}$ M. Three ml per sample of fluorescein-labeled BSA ~~(BSA-FL_9)~~ (BSA-FL_9) at concentrations ranging from 10^{-10} M to 10^{-7} M was injected into the flow cell. The injection was performed over a five-minute interval. The spectrum at wavelength of 488 nm was taken and the bulk BSA-FL_9 was removed by flushing with PBS buffer. Three more spectra

were taken, and the fluorescein peak from 513 to 517 nm was integrated. These values were set versus the log of BSA-FL_9 concentration in order to obtain the binding isotherm.

Please amend paragraph number **[0119]** as follows:

[0119] The levels of nonspecific absorption of antigen on waveguides prepared by ~~site-specific~~ site-specific coupling with avidin-biotin (Example II; Table I, rows 7 and 8 from the top) or hydrogel (Example I; Table I, bottom two rows) were considerably better than most of the prior art coupling methods, being typically 1-3% (Table I). The results also indicated that nonspecific binding to the avidin-coated waveguide was acceptably low for analyte molecule concentrations of less than about 10^{-5} M, without a wash step.

Please amend paragraph number **[0121]** as follows:

[0121] The row labeled silica-avidin with biotin-PEG represents data obtained with the further refinement of preloading the surface (after attachment of the capture molecules) with ~~biotin-PEG~~ biotin-PEG conjugates. This was done to passivate potential nonspecific binding regions. However, the improvement obtained with biotin-PEG preloading was not large.

Please amend paragraph number **[0124]** as follows:

[0124] The row labeled silica-avidin with biotin-PEG represents data obtained with the further refinement of preloading the surface (after attachment of the capture molecules) with ~~biotin-PEG~~ biotin-PEG conjugates. This was done to passivate potential nonspecific binding regions. However, the improvement obtained with biotin-PEG preloading was not large.

Please amend paragraph number **[0127]** as follows:

[0127] In the experiments whose results are presented in Tables II and III, the specific binding values were determined using hCG labeled with ^{125}I, as described for Table I, while ~~^{125}I~~

~~labeled~~ ^{125}I-labeled BSA was used to measure the nonspecific binding. In both Tables II and III, the immobilized antibody was anti-hCG-A.

Please amend paragraph number **[0128]** as follows:

[0128] The fluoroimmunoassays of FIGS. 7A, 7B, 8, 9A-9F, and 10A-10D and Tables I-III were performed using an interfacial fluorometer constructed at the University of Utah. Silica waveguides with the appropriate respective immobilized antigens were placed in the dual-channel flowcell of FIGS. 3A and 3B. The two channels were used for sample and reference measurements, as described with respect to FIGS. 4A-C. The light source was the 514.5 nm emission of an ~~air-cooled~~ air-cooled argon-ion laser. The laser beam was split into two parallel beams, which were focused with lenses into the two channels of the waveguide. Fluorescence emission was recorded from 520 to 620 nm using a monochromator connected to a computer-controlled CCD camera. The fluorescence spectrum was integrated from 560 nm to 600 nm to improve the signal-to-noise ratio.

Please amend paragraph number **[0130]** as follows:

[0130] For purposes of the tests shown in FIGS. 7A, 7B and 8, the antibody to be detected (the analyte) was chosen to be a monoclonal antibody (designated anti-hCG-A) to an hCG antigen (the latter designated hCG-A). The data of FIG. 7A were obtained with whole hCG molecules serving as the capture molecules (the antigen or analyte binding molecule) in the assay. The data of FIG. 7B were obtained using an oligopeptide constructed to selectively bind the ~~anti-hCG-A~~ anti-hCG-A antibody as the capture molecules. Oligopeptides suitable for this purpose for any known antigenic analyte molecule analyte can be obtained using the methods of Geysen et al. as disclosed in Patent Publication No. WO 86/86487 and U.S. Patent No. 4,708,871, as well as in the scientific literature. To attach the necessary fluorescent dye, either the N-terminus of the oligopeptide was modified to provide an amino group for amino-reactive dyes, or the C-terminus was modified to provide a cysteine thiol group for thiol-reactive dyes. Preferably also, the

complete oligopeptide sequence is of length sufficient that the attached dye is spaced from the binding site by at least two or three residues.

Please amend paragraph number **[0137]** as follows:

[0137] FIGS. 9A-F depict results obtained with different pairwise combinations, with Fab' fragments prepared from anti-hCG-A (Fab'-A) and immobilized to waveguides using the ~~avidin-biotin~~ avidin-biotin coupling chemistry. Fab' fragments prepared from anti-hCG-B, anti-hCG-C and anti-hCG-D were labeled with tetramethylrhodamine for use as tracer antibodies (designated Fab'-B, Fab'-C and Fab'-D, respectively). FIGS. 9A and 9B show results with Fab'-B as the tracer molecule. FIGS. 9C, 9D show results obtained using Fab'-C as the tracer molecule. FIGS. 9E and 9F show results obtained using Fab'-D as the tracer molecule. Presently, Fab'-B and Fab'-C are preferred for use as tracers in an hCG assay.

Please amend paragraph number **[0139]** as follows:

[0139] FIGS. 10A-D show data obtained from a competition or displacement assay. ~~Fab'-A~~ Fab'-A fragments were immobilized to waveguides using either the avidin-biotin chemistry (FIGS. 10A, 10B) or the hydrogel coupling chemistry (FIGS. 10C, 10D). The immobilized Fab'-A fragments were preloaded with the tracer oligopeptide at a concentration of 10^{-8} M. Increasing concentrations of hCG were added to one channel of the flow cell (sample) and PBS buffer was added to the other (reference). For each coupling chemistry, the raw fluorescence intensities of the sample and reference channels are shown in the panels on the left (10A & 10C) and the percent of full-scale fluorescence (in the absence of hCG) is shown in the panels on the right (10B & 10D). The latter values were normalized for the change in reference fluorescence. Standard errors were plotted for all data points, but in some cases were smaller than the plot marks.

Please amend paragraph number **[0144]** as follows:

[0144] Also, while the present description is primarily with reference to ~~PLURONICS-type~~ PLURONICS-type compounds, it is within contemplation that other polymeric compounds having hydrophilic segments and hydrophobic segments and offering pendant OH groups for attachment of proteins or photo-activated linkers will be useful. As known in the art, these include SEPHAROSE-type materials and other polysaccharides. Also, block copolymers having polyurethane segments as the hydrophilic block may be useful.

Please amend paragraph number **[0145]** as follows:

[0145] Referring to FIG. 16, a general procedure for preparing a patterned polystyrene waveguide is as follows. First, a waveguide surface 700 coated with PF108 molecules 702 is prepared. Next, the free PEO chain ends 704 of the PF108 molecules 702 in a selected region of the waveguide are derivatized in a photo-activated coupling reaction with a photoaffinity crosslinker 706. Suitable crosslinkers are heterobifunctional reagents which have a photo-activatable group conjugated to a reactive functional group such as isothiocyanate, succinimide or maleimide. Upon irradiation with light beam 701 of the appropriate wavelength (generally in the ultraviolet region), the photo-activatable groups of the crosslinker 706 react to covalently bind to the free PEO chain ends 704. A mask 712 (FIG. 15) confines the irradiation to a first region 714 of the waveguide. The result is a waveguide surface having reactive functional groups useful to bind Fab' fragments only in the first region 714. Next, the waveguide surface 700 is incubated with a solution of Fab' fragments of a first species ~~(FAB 1~~ (Fab' 720 in FIG. 16) for a time sufficient to allow the binding of Fab' fragments to the derivatized region to go to completion. The unreacted Fab' fragments are then washed off, and the process of photo-activated derivatization is repeated for a second region of the waveguide, followed by incubation with a second species of Fab' fragment.

Please amend paragraph number **[0148]** as follows:

[0148] Suitable photoaffinity crosslinkers include aryl azides (amine-to-amine linkage), fluorinated aryl azides (C-H bond-to-amine linkage), and benzophenones (C-H ~~bond-to-amine~~ bond-to-amine linkage or C-H bond-to-thiol-linkage, depending on the specific compound). Examples of each type are shown in FIGS. 18A-18D, along with the corresponding photo-activated coupling reaction. Presently, benzophenones providing a C-H bond-to-thiol linkage are preferred, as these can be used to achieve site-specific coupling to Fab′ fragments. Either the iodoacetamide or the maleimide derivatives of benzophenone ("BPIA" and "BPM," respectively) can achieve this purpose. At present BPM is preferred, as it exhibits a higher degree of specific binding and a lower degree of nonspecific adsorption. This is because the coupling occurs via the C-terminal thiol groups of the Fab′ fragments, as described previously herein for the PMahy coating. Other photo-affinity crosslinkers providing free maleimido groups may be equally suitable.

Please amend paragraph number **[0149]** as follows:

[0149] Table IV shows comparative data on the levels of specific binding and nonspecific binding obtained for the crosslinkers BPM vs. BPIA for surfaces which are uncoated or coated with one of four different TBCPs, and for the procedure of FIG. 16 vs. that of FIG. 17. The TBCPs are PF108 and three others designated by the tradenames PP105, PF68, and PF88, also available from BASF. The respective PEO/PPO/PEO ratios and molecular weights of these compounds are 37/56/37 (PP105, MW=6500), 76/30/76 (PF68, MW=8400), and 104/39/104 (PF88, MW= 11,400). As a model system, Fab′ fragments derived from the 9-40 anti-fluorescein antibody were used as the capture molecules, with fluorescein-conjugated BSA representing the analyte. The BSA was radioactively labeled. Specific binding was determined as the binding of the ~~fluorescein-BSA-conjugate,~~ fluorescein-BSA-conjugate, while nonspecific binding was determined from binding of native (unconjugated) BSA.

Please amend the paragraph notation below Table IV (appearing in the middle of paragraph number **[0149]**) as follows:

> In all experiments except Nos. 14 & 15, the substrate was a polystyrene surface. In expts. 1-3, there was no PLURONICS coating. In experiments 4-13, the surface was coated for 24 hours with a 4% w/v aqueous solution of the indicated PLURONICS compound. In experiments 1, 3, 4, and 6-15, the Fab' was coupled first to the crosslinker and the complex then photo-crosslinked to the substrate. In experiments 2 and 5, the crosslinker was first photo-coupled to the surface, then incubated with ~~Fab.~~ Fab'. The concentration of crosslinker used was a 20-fold molar excess of the Fab' concentration.

Please amend paragraph number **[0154]** as follows:

[0154] The above-described coupling scheme is very effective with a hydrophobic substrate such as a polystyrene waveguide, but less useful with silica-based substrates such as quartz, glass, and other silicon-based optical materials. Therefore, in an alternate embodiment of the photocoupling method for a waveguide made of a silicon-based material, a silica surface is treated with an undercoating to which a thin top coating of PEG polymer (the protein-resistant component) will effectively adhere. Three schemes for accomplishing this are described in detail herein; all use an undercoating which is a silica-affinic agent having a silyl group free to react with silica. The first scheme is described previously herein in Examples I and III. The second scheme uses avidin to couple a biotinylated-PEG to the surface. This scheme is similar in some respects to Example II, but is modified as described in Example IV. The third scheme is to use an undercoating which makes the silica surface hydrophobic (such as DDS, dichlorodimethylsilane, or DPS, diphenyldichlorosilane), and then to use one of the block copolymers as the top coating. Still another embodiment for use with silica surfaces employs a single coating of a silyl-modified PEG such as methoxy-poly(ethyleneglycol) trimethoxysilane ("PEG-silane") of molecular weight around ~~3500-5000.~~ 3500 to 5000.

Please amend paragraph number **[0155]** as follows:

[0155] The photo-linking process described above with reference to polystyrene surfaces can be adapted for any of these four silica coating schemes, preferably using a benzophenone ~~photo-linker~~ photo-linker as described in reference to FIGS. 16 and 17 (Example IV). Similar considerations of the relative and/or absolute levels of nonspecific binding apply in selecting preferred undercoating/top coating combinations. Example IV and Table VI describe experiments and results of several such combinations for use with silica-based substrates.

Please amend paragraph number **[0156]** as follows:

[0156] The specific and nonspecific binding properties of four kinds of coated silica surfaces (silica-MSil(5000), silica APS-Glu-PEG(2000), silica-avidin-biotin-PEG(3400), and ~~silica-DPS-PF108),~~ silica-DPS-PF108),to which Fab′ fragments were photo-crosslinked with BPIA, were evaluated. MSil(5000) is the trimethoxysilane derivative of methoxy-PEG(5000), where PEG(5000) is polyethylene glycol of molecular weight approximately 5000. Diphenylsilane dichloride (DPS) is a generally hydrophobic compound which has an $SiCl_2$ group which can react with the Si-OH bonds in silica.